

Research Note

Morphological Observations on Third-Stage Larvae of *Anisakis simplex* A (Anisakidae: Nematoda) from Adriatic and Ionian Waters

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ABSTRACT: Additional morphology on third-stage larval specimens of *Anisakis simplex* A (Rudolphi, 1809, det. Krabbe, 1878) infecting *Merluccius merluccius* L. and *Sardina pilchardus* Walb. from the Adriatic and Ionian seas (Southern Italy) is described and illustrated. Particular attention (light, scanning electron microscope observations, and histological studies) was given to illustrate head structures such as papillae, oral and amphidial openings, excretory pore, boring tooth, excretory system, rectal glands, and tail.

Larvae collected in Mediterranean waters were morphologically similar, and morphometrics fit well (with considerable overlap in most measurements) with the previous descriptions of *A. simplex* A (type I larvae)

reported from Australian, Canadian, Japanese, North Sea, northeast Atlantic, and New Zealand waters, confirming its cosmopolitan geographical distribution.

KEY WORDS: *Anisakis* larvae, Mediterranean Sea, *Merluccius merluccius*, *Sardina pilchardus*, SEM morphology.

Seventeen species of the genus *Anisakis* Du-jardin, 1845 (Nematoda: Ascaridata), have been studied in detail by Davey (1971), showing that spicules, postanal papillae, form of ventriculus, vulva position, and lips shape are the main char-

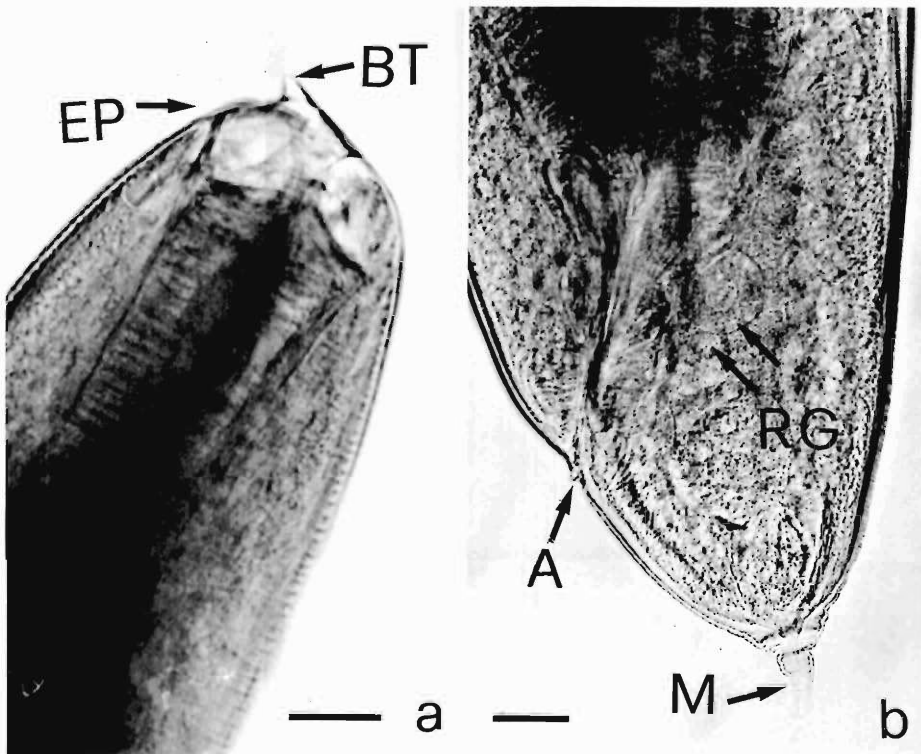


Figure 1. *Anisakis simplex* larvae (L3). a) Anterior extremity, lateral view (BT = boring tooth, EP = excretory pore). b) Posterior extremity (A = anus, M = tail spine [mucron], RG = rectal glands). Scale bar = 25 μ m.

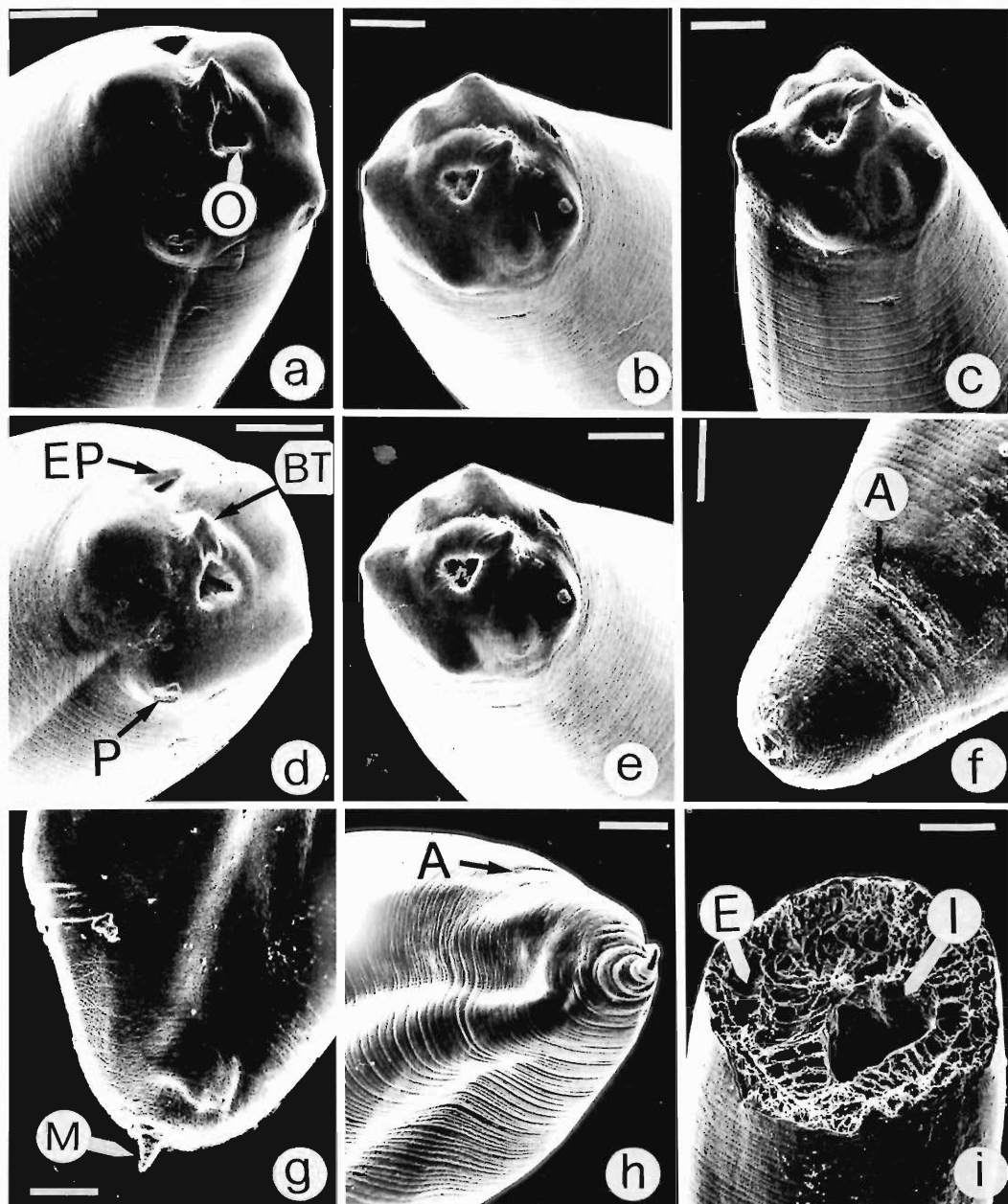


Figure 2. SEM morphology of Mediterranean *Anisakis simplex* larvae (L3). a–e) Anterior end en face view or profile showing oral (O) opening, papilla (P), and boring tooth (BT). f–h) Ventral and lateral view of the posterior end (A = anus, M = tail spine). i) Cross-section at 10% of body length showing the intestine (I) and the excretory cell (E). Scale bars: a–h = 40 μ m; i = 100 μ m.

acters, in order of importance, for identifying adult specimens of *A. simplex* (Rudolphi, 1809, det. Krabbe, 1878), *A. typica* Diesing, 1860, and *A. physeteris* Baylis, 1923. Morphological illustration of larval stages is less extensive because

of their uncertain identification and the difficulties in following their life cycle.

Last year in Southern Italy, extensive alarm for public health was raised by the occurrence of *Anisakis* sp. larvae found in the peritoneal cavity

Table 1. Measurements of *Anisakis simplex* A larvae from *Merluccius merluccius* and *Sardina pilchardus* ($n = 25$).

	$\bar{x} \pm SD$	Range
Body length (mm)	21.60 \pm 3.47	15.00–27.50
Body width (mm)	0.41 \pm 0.05	0.34–0.51
Esophagus length (mm)	2.65 \pm 0.29	2.06–3.08
Ventriculus length (mm)	0.70 \pm 0.08	0.59–0.92
Ventriculus width (mm)	0.24 \pm 0.05	0.15–0.32
Tail (mm)	0.11 \pm 0.01	0.09–0.14
Anal body width (μ m)	119.00 \pm 17.66	69.33–150.67
Tail's mucron (μ m)	25.69 \pm 4.15	17.33–32.00
Boring tooth (μ m)	9.35 \pm 2.41	5.50–14.50
a	52.91 \pm 5.10	42.86–59.46
b	8.15 \pm 1.05	6.80–11.16
c	199.93 \pm 43.49	133.3–288.9
c'	0.95 \pm 0.21	0.78–1.79

of *Merluccius merluccius* and *Sardina pilchardus*, common fish species of the South Adriatic and Ionian seas.

The third-stage larval (L3) *Anisakis* nematode population from Mediterranean waters was identified as *A. simplex* A (type I larvae; Berland,

1961) from ventriculus dimensions and the presence of the tail spine (mucron), according to the key suggested by Pippy and Van Banning (1975).

Orecchia et al. (1986), Nascetti et al. (1986), and Beverley-Burton et al. (1977) have proposed the use of multilocus electrophoresis to provide diagnostic characters for the identification of larvae of the *Anisakis simplex* complex from the Mediterranean Sea and northeast Atlantic. Nascetti et al. (1986) also suggested, on the basis of biochemical data, to synonymize *A. simplex* A with *A. pegreffi* (already synonymized with *A. simplex* by Davey [1971]). Orecchia et al. (1989) reported later the occurrence of larvae of *Anisakis* sp. from Italian waters, identifying them as *A. simplex* A (type I larvae) and *A. physeteris* (type II larvae), using biochemical keys, without giving morphometrical features.

Here a morphological and morphometrical illustration of the Mediterranean population of *A. simplex* A is presented and compared to those reported from Australia, the North Sea, New Zealand, and Japan (Brunsdon, 1956; Koyama et al., 1969; Pippy and Van Banning, 1975; Smith, 1983; Hurst, 1984).

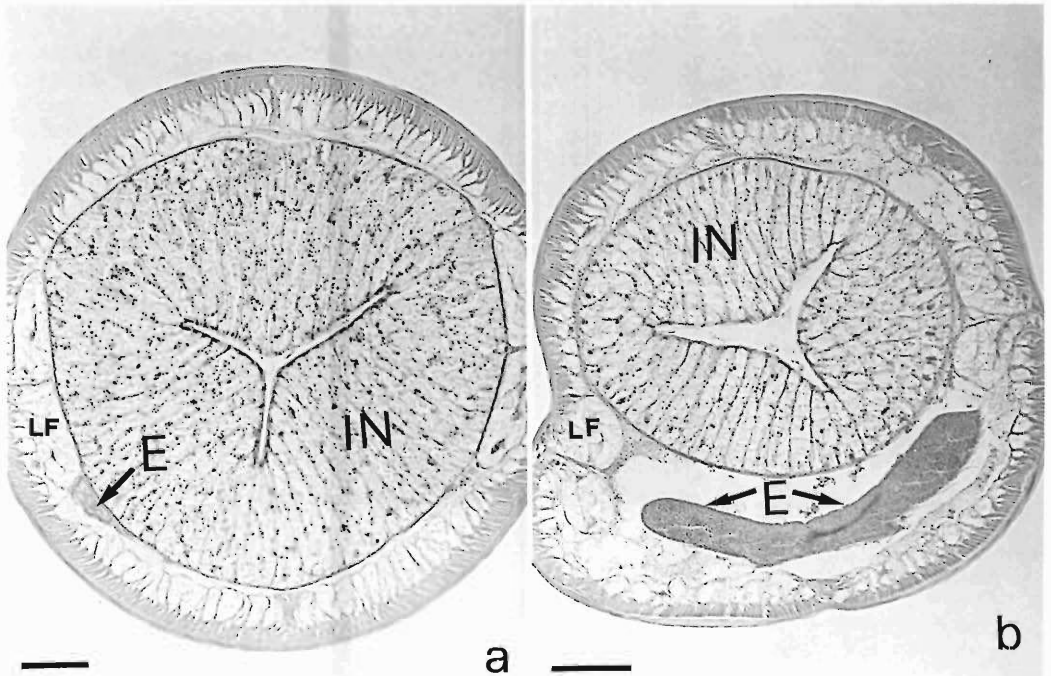


Figure 3. Cross-sections at 8% (a) and 35% (b) of body length of Mediterranean *Anisakis simplex* larvae showing the different shape and size of the excretory cell (E) within the body. IN = intestine, LF = lateral fields. Scale bar = 40 μ m.

Table 2. Measurements of *Anisakis simplex* A larvae from teleosts.

Locality	Authority	n	BL (mm)		%BW/BL		%EL/BL		%VL/BL		%TL/BL	
			$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range	$\bar{x} \pm SD$	Range
North Sea	Pippy and Van Banning, 1975	20-24	19.7	16.1-22.5	2.48 \pm 0.48	—	13.3* \pm 2.41	—	—	—	0.66 \pm 0.14	—
	Punt, 1941	22-56	20.4	13.3-30.0	1.78 \pm 0.39	—	12.2* \pm 2.02	—	—	—	0.53 \pm 0.15	—
Northeast Atlantic Australia	Smith, 1983	30	22.6	—	—	—	—	—	4.10	—	—	—
	Brundson, 1956	10	20.6	15.9-25.0	2.19 \pm 0.12	—	9.84 \pm 1.05	—	3.05 \pm 0.27	—	0.54 \pm 0.07	—
	Cannon, 1977	60	19.7	14.6-24.6	—	—	—	—	—	—	—	—
	Hurst, 1984	50	20.3 \pm 3.04	14.0-26.0	2.14 \pm 0.21	—	9.99 \pm 1.50	—	3.43 \pm 0.50	—	0.59 \pm 0.12	—
Chile	Torres et al., 1978	7	28.2	19.7-39.3	2.13	—	8.34	—	3.84	—	0.51	—
Japan	Koyama et al., 1969	139	28.4	19.0-36.0	1.58	—	7.79	—	3.92	—	0.40	—
	Shiraki, 1974	9	28.4	23.0-31.7	1.73 \pm 0.26	—	7.2 \pm 0.26	—	4.59 \pm 0.94	—	0.40 \pm 0.05	—
Mediterranean Sea	This study	25	21.6 \pm 3.47	15.0-27.5	1.91 \pm 0.20	1.68-2.33	12.5* \pm 1.47	8.96-14.70	3.30 \pm 0.40	2.84-4.00	0.52 \pm 0.12	0.35-0.75

* Esophagus including ventriculus.

Material and Methods

Specimens were collected during spring 1992 from the peritoneal cavity of *Merluccius merluccius* and *Sardinia pilchardus* in various localities of the South Adriatic and Ionian seas, with a prevalence of 39 and 31% and a range of intensity of 1-25 and 1-6 for *M. merluccius* and *S. pilchardus*, respectively ($n = 100$ of each species). Our data regarding prevalence and intensity for *M. merluccius* are quite close to those reported by Orecchia et al. (1989).

Nematodes for light microscope studies were fixed in 4% formaldehyde solution and mounted permanently in dehydrated glycerine following Seinhorst's (1959) method. Specimens for scanning electron microscopy (SEM) were processed by Eisenback's (1985) method and observed with a JEOL 50-A stereoscan. Glycerine-infiltrated specimens were also used for SEM observations.

For histological studies, specimens were fixed in Bouin's solution, dehydrated in ethanol, and embedded in Histowax. Transverse (cross) sections were cut at 5 μ m and stained with hematoxylin-eosin.

A comparison of all previous descriptions of populations of *A. simplex* A from the North Sea, northeast Atlantic, Australia, New Zealand, and Japan to those of the present study was also made, using the following morphometrical parameters: BW/BL (body width/body length), EL/BL (esophagus length/body length), VL/BL (ventriculus length/body length), and TL/BL (tail length/body length), expressed as a percentage according to Hurst (1984). Body ratios were also calculated (Siddiqi, 1986): a (body length/body width), b (body length/esophagus length), c (body length/tail length), and c' (tail length/body width at anus).

Specimens of the Mediterranean population of *A. simplex* A L3 are deposited in the collection of the Museum National d'Histoire Naturelle, Paris, France, and several Bouin's fluid-fixed specimens are deposited in our institute.

Results

L3s ($N = 25$) obtained from either fish species are morphologically and morphometrically identical (Table 1). The cuticle is 12-16 μ m thick, usually with distinct striations mainly at the anterior and posterior body extremities (Figs. 1, 2). In en face view (SEM observation), a triangular oral opening is visible between trilobed lateral lips; a prominent V-shaped projecting boring tooth (3-9 μ m long) is located ventrally to the mouth. The excretory opening, seen by light microscope below the boring tooth on the ventral side, is revealed by SEM as an oval lateral slit (Fig. 2). Rectangular to circular outlines of papillae could be seen on each of the lateroventral lips (Fig. 2). Cross-sections of the excretory cell along the body of larva show that it has different shapes and dimensions at different body levels (Fig. 3) and is always contiguous to hypodermal cells, the alimentary canal, and sometimes the

somatic musculature. At the pharynx level, it has an oval section, a well-visible central duct and appears full of eosinophilic granulation. At mid-body, it increases enormously, its lobes enveloping the ventral portion of the intestine; posteriorly it becomes narrower and ends just before the anal opening. The short tail (Table 1) ends with a distinct, not reflexed, mucron (Fig. 2). There are 3 rectal glands: 2 are dorsal and 1 is ventral (Fig. 1b), well visible on glycerine-mounted specimens.

REMARKS: The L3 *Anisakis* larvae from the Adriatic and Ionian seas are very close (with considerable overlaps) to the type I larvae found by Brunson (1956) in 54 New Zealand fishes and fit very well with all the previous descriptions (Berland, 1961; Koyama et al., 1969; Table 2) of this larval stage.

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